




# THE RODENT SEMINAR SERIES



presented by the Research Animal Resources (RAR)  
the JHU Animal Care and Use Committee (ACUC) Office,  
And Transnetyx



## Seminar Details

- GOAL: Enhance education and training, and compliance
- Every 4th Wednesday of the month from 3-4 PM
  - Same Zoom details for the entire seminar series
  - In-person location – may vary
- Attendance recorded
  - Through Zoom report or attendance sheet here
    - If you are on Zoom and your full name is not on display, please email Jason Villano your full name or write it on the chat board
- Prizes
  - 3 individual prizes
  - 3 lab prizes



# RODENT BREEDING COLONY MANAGEMENT: BEST PRACTICES

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## Seminar Overview:

- **Part I – RAR + ACUC**
  - Basic Anatomy and Physiology
  - Reproductive Parameters
  - Sexing Techniques
  - Genotypes, phenotypes, and nomenclature
  - Planning: Breeding Considerations
  - Researcher Responsibilities
  - Breeding Schemes
  - Genetic Fundamentals and Basic Breeding Systems
  - Management of Breeding Cages
  - Weaning and Caging Density
  - Factors Affecting Production
  - Common Issues and Troubleshooting
  - JHU ACUC Policies and Guidelines
- **Part II – Transnetyx**
  - Breeding Efficiency Using Software
  - Implementing Best Practices
  - Colony Management Software Overview
  - Planning Production
  - Key Factors

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## Why should you care?



- Breeding management can affect the **health and welfare of the animals and the quality of your research!**
- **Efficiency – time and cost saving**
- **Poor management strategies -> potential confounding variable for research outcomes**
- **Compliance**



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## Basic Anatomy and Physiology



- Adult weights: male 20 - 40g, female 18 - 35g
- Newborn weight: 1 – 1.5g
- Typical weaning weight: ~10 g
- Sexual maturity: male 5 – 7wks, female 4 – 5wks
- Life span 1 – 3 years
  - Reproductive performance declines at ~8-10 months
- Daily water consumption: ~1.5 ml/10g BW
- Daily food consumption: 1.2 – 1.8g/10g BW
- Female="dam", male="sire", offspring="pups"

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## Reproductive Parameters



- Polyestrous
  - Estrus cycle: 4 - 5 days
- Postpartum estrus (14-28hrs post parturition)
- Gestation: 19 - 21 days
- Number of teats: 10 (5 pairs)
- Average litter size: 4 – 14
- Pups eat solid food: P11-P12 (P=postnatal day)
- Weaning age: P21 - P28 days

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## Sexing Techniques



- Several methods:
  - Visual inspection of anogenital distance
    - Longer in males
  - Visual inspection of teats (~P8-P10)
    - May be more challenging in white mice
  - Genotyping
- **Important!!!**
  - Incorrect sexing -> unplanned inbreeding, inappropriate breeding schemes, fighting

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## Sexing: Visual Inspection

Anogenital distance is longer in males.

- Red circles- anus
- Yellow circles- genital papilla
- Blue circles- teats on female

Male Female

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## Genotypes, phenotypes, nomenclature

- **Genotype:** The complete set of genetic information carried by an individual.
- **Phenotype:** The observable or measurable characteristics of an organism or individual.
- **Nomenclature:**
  - International Committee on Standardized Genetic Nomenclature for Mice
    - Establish rules & guidelines for official names of genes, alleles, and strains
  - Mouse Genome Informatics Database
  - Mouse Phenome Database
  - Rat Genome and Nomenclature Committee

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## Planning: Breeding Considerations

- Assess experimental needs prior to initiating breeding
  - How many mice will you need? How many of each sex?
  - Which mice do you need to genotype and when?
  - How will experimental needs change over time?
    - Fluctuations in reproductive vigor over time
    - Consider cryopreservation of
      - embryo or sperm
      - save space, time, reassurance
- Availability of breeders
  - Experimental and breeder stocks

The Johns Hopkins Transgenic Core

- Services Include:
- Sperm Cryopreservation
- Embryo Cryopreservation
- IVF
- Embryo Rederivation
- And more!

Contact: Chip Hawkins  
chawkins@jhmi.edu

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## Researcher Responsibilities

- **Genotyping**
  - Important to routinely genotype mice to maintain consistency within mouse colonies
  - Sample type: tail (5mm max), ear punch, blood, fecal pellet, mucosal cells
  - Ensure hemostasis *before* returning animals to cage
  - Timing: at or before weaning
    - **NOTE: Tail snip after P21 -> local or general anesthesia is required**

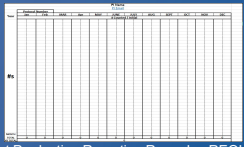
**ACUC Guidelines on Tail Biopsy of Mice**  
<https://web.jhu.edu/animalcare/policies/Tail%20Biopsy%20of%20Mice.pdf>

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## Researcher Responsibilities



- **Identifying**
  - Ear tags, tattoos, ear notch, tail marking, toe clip (conditional), microchip
- Tip #1: As a refinement, you can do ear notching for genotyping and use that as identification method as well.
- **Recordkeeping**
  - Pedigree records, production records, *production reporting*
  - Mating dates, birth dates, litter indices (# born, # weaned, sexes of offspring, weights), weaning dates



Rodent Production Reporting Records - REQUIRED

**Johns Hopkins University  
Animal Care and Use Committee**

**Ear Tagging of Mice?**

REVISION: This guideline provides instructions for the proper placement of ear tags.

**INTRODUCTION:**

• Animal research personnel involved with the management of animal research facilities are responsible for identifying and tracking their mice and breeding them for use in research. Ear tags are a critical component of animal research facilities and are used to identify individual mice for research purposes. Ear tags are used to identify individual mice for research purposes and are used to identify individual mice for research purposes.


**PROCEDURE:**

• The ear tag should be placed on the ear of the mouse. The ear tag should be placed on the ear of the mouse. The ear tag should be placed on the ear of the mouse. The ear tag should be placed on the ear of the mouse.

**REVISIONS:**

• This document is for informational purposes only. It is not intended to be used as a substitute for professional advice. It is not intended to be used as a substitute for professional advice. It is not intended to be used as a substitute for professional advice.

Microtagging of Mice




Contact the ACUC for the tattoo training

See complete ACUC Guideline on Ear Tagging of Mice at: <https://web.jhu.edu/animalcare/policies/>

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## Breeding Schemes



- **Monogamous: 1 ♂ : 1 ♀**
  - Usually paired for duration of reproductive lifespan
    - Female productivity maximized
  - Pros: easiest to manage (recordkeeping, parentage), space->postpartum estrus
  - Cons: less space efficient, allomaternal care does not occur, requires more animals
- **Polygamous: Trio(1 ♂ : 2 ♀) vs Harem(1 ♂ : 3+ ♀)**
  - Pros: efficient use of male (i.e., when availability of males is limited) to produce larger quantities of offspring/male. Allomaternal care can occur in cases of maternal rejection.
  - Cons: more work, risk overcrowding, separate females from harem when pregnant, may miss postpartum estrus

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## Management of Breeding Cages



- **Pair breeders early**
  - Mate early (~6-12 weeks of age)
- **Retire breeders at ~7-8 months of age**
  - Timing of replacement will depend on age and strain/stock-specific reproductive vigor
    - Longer for good breeders and males
- Tip # 2: Post-partum estrus can be used to increase breeding colony production
  - wean current litter just before delivery of the gestating litter
- **Wean at ~ 21 days of age (or ~10 g BW)**
- **Monitor cages with litters frequently**
  - Separate multiple litters to avoid overcrowding and trampling
- **Document changes within your colony**
  - Record everything!

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## Caging Density




- Do not overcrowd cages
  - Overcrowding compromises the health and welfare of mice in cage
- **Adults – 5 max/cage**
- **Weanlings – up to 9/cage**
  - Must reduce numbers to 5/cage by 6 wks of age
- **Include DOB or age on cage card**
  - No age listed on cage card = 5 max./cage

Note: Cage Density is currently under review.

See full ACUC Overcrowded Cage Policy at: [https://web.jhu.edu/animalcare/policies/Overcrowded\\_cage\\_policy\\_April\\_2014.pdf](https://web.jhu.edu/animalcare/policies/Overcrowded_cage_policy_April_2014.pdf)

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## Factors Affecting Reproduction



- **Environmental variations**
  - Microenvironment vs Macroenvironment
  - Noise and vibration
  - Temperature and humidity
    - Enviropak™, Nestlets™->thermoregulation
  - Waste gas pollutants
  - Odors (perfumes, pheromones)
  - Light cycles and intensities
- **Diet**
  - Obesity
  - Breeder chow
- **Maternal variables**
  - Parity Status
- **Strain/stock**
  - Outbred>inbred
- **Health**

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## Factors Affecting Reproduction



- **Cage composition and pheromones may affect breeding**
  - Whitten Effect
    - Synchronization of the female estrus cycle ~72h after a group of female mice is introduced to a male or his odor
  - Bruce Effect
    - Female mice spontaneously abort when exposed to a male or his odor if he was not their original mate
  - Lee-Boot Effect
    - Induction of pseudopregnancy in group-housed females
  - Vanderbergh effect
    - Early induction of the 1st estrus in prepubertal female as a result of exposure to pheromone-laden urine of sexually mature male
- **These effects are important to consider when breeding, especially for timed mating**
  - E.g. for the creation of transgenic mice or for cryopreservation

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## Common Issues



- **No litters are being produced**
  - Might be due to several factors
    - e.g., age of breeders, genotype and phenotype, environment
  - **Troubleshooting:**
    - Assess breeding records
    - Assess strain/stock phenotypic expected reproductive vigor
    - Use proven stud male if available
    - Consider dietary changes (especially if experimental restrictions)
      - Tip # 3: Supplements like Love Mash™ (Bio-Serv) and sunflower seeds might help.





Figure 1: One supplement that has shown effectiveness in improving breeding in difficult strains is Love Mash™.


Love Mash is a high-fat supplement specifically formulated for breeding mice.

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## Common Issues



- **Pups dying**
  - Cannibalism and maternal rejection
  - More common with new breeders, primiparous dams, overcrowded cages
  - Increased frequency if litter is disturbed too early/often
  - **Troubleshooting:**
    - Plan experimental manipulations to minimize handling
    - Assess strain/stock phenotypic expected reproductive vigor
      - Caveat: separate dam + litter to avoid overcrowding
    - Consider use of shelter and nests
    - Consider communicating with care staff regarding cage changing
    - Consider dietary changes (especially if experimental restrictions)



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## Common Issues


- **Fighting**
  - More common between adult males
    - Especially adult males from different litters
  - More common w/ inappropriate breeding schemes
    - Example: 2+♂ : ≥1♀
  - **Troubleshooting:**
    - Recheck sex of mice in cage
    - Ensure appropriate breeding scheme, housing density
      - Separate adult males from breeding cages
    - Report fight wounds to RAR staff.



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## Common Issues


- **Dystocia:** complicated, prolonged, or difficult birth
  - Mice usually deliver at night and in a couple of hours
  - Long labor or delivery during the day take a closer look
    - Signs: Vaginal bleeding, pups stuck in birth canal, decreased activity/lethargy seen in dams that are due to give birth
  - **Troubleshooting:**
    - Monitor dams that are due to give birth frequently and in AM
    - **Place emergency clinical call immediately or euthanize**
      - RAR veterinary staff may be able to provide medical and/or surgical intervention
      - *Important: include lab point of contact on cage card!!!*



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## Common Issues

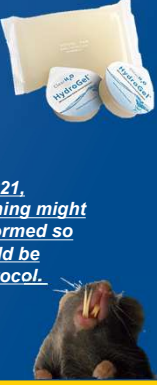
- **Weaned too small**
  - Standard weaning at ~P21 (~10 g BW)
  - May occur if weaned too young
  - Some strains/stocks mature slower and are smaller at P21
    - May not be able to eat/drink on its own



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## Common Issues

- **Weaned too small**
  - **Troubleshooting:**
    - Document/record birth dates to ensure not weaning too young
    - Add diet gel and hydrogel to cage of newly weaned animals
    - Assess for malocclusion
    - **Tip # 4: If pups are typically less than 10 g of body weight at P21, this might be due to the phenotype. In this case, delayed weaning might be considered, but post-partum estrus mating cannot be performed so as to prevent overcrowding. Intended weaning schedule should be described in your Breeding Colony Form with your ACUC protocol.**



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## Common Issues

- **Congenital and inheritable abnormalities**
  - May be present at birth or develop overtime
  - May be a result of inbreeding or strain-related
  - **Troubleshooting:**
    - Consult with RAR veterinary staff
    - Consider mouse phenotype
    - Consider not breeding affected animals
    - NOTE: Euthanasia might be needed for affected animals.



Microphthalmia



Hydrocephalus

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## Summary of Key Points

- Plan ahead
- Monitor outcomes
- Record everything!
- Consider common issues and contact RAR for troubleshooting breeding issues.
- Tip # 5: RAR manages a breeding core and provides rodent breeding services.
- Report clinical issues.




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## JHU ACUC Guidelines

- Multiple guidance documents and instructions
  - Mouse Breeding Guidelines
  - Overcrowded Cage Policy
  - Euthanasia of Rodents using CO2
  - Ear Tagging of Mice
  - Documenting the Number of Mice Produced by Researchers at JHU
  - Tail biopsies
  - Toe Clipping
  - **Rat Housing Density**

*"Documents included with reference packet provided with presentation"*

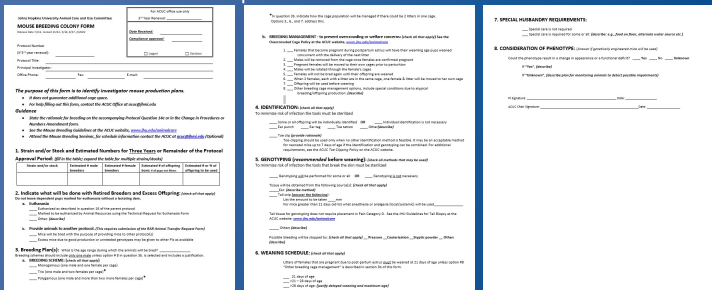
<https://web.jhu.edu/animalcare/policies/index.html>



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## JHU ACUC Guidelines Continued

- Mouse breeding colony form
  - Submitted with ACUC protocol




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## JHU ACUC Guidelines Continued



Overcrowded Cage Policy

- Item 7: "Timeline for separating"
  - Laboratory personnel must separate an overcrowded cage within 2 days of being notified. However, a severely overcrowded cage must be separated within 4 hours or sooner if indicated by the conditions.
- Item 9: Chronic Overcrowding
  - Chronic failure of a laboratory to respond to RAR's request to separate overcrowded cages, or removal of "Overcrowded" tape without separating the cage will be referred to the Animal Care and Use Committee (ACUC) as a compliance concern.

 **Johns Hopkins University**  
Animal Care and Use Committee

**Research Animal Resources (RAR)' Policy and Procedures for Handling Overcrowded Mouse Cages\***

**Purpose:**


1. To provide operating procedures for the animal care staff for use in the event of cage overcrowding.
2. To provide information to mouse researchers regarding the rules of cage population management.

**Note:** The expectation is that lab personnel who manage breeding colonies will keep track of the colonies' weaning and separation needs. While the animal care staff play a role in alerting researchers when a cage has become overcrowded, it is reserved only to be backup plans to keep the university in compliance with regulations. It is the responsibility of the researcher to keep up with their colony management.


See full ACUC Overcrowded Cage Policy at:  
[https://web.jhu.edu/animalcare/policies/Overcrowded\\_cage\\_policy\\_April\\_2014.pdf](https://web.jhu.edu/animalcare/policies/Overcrowded_cage_policy_April_2014.pdf)

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## JHU ACUC Guidelines Continued



- Documenting the Number of Offspring Produced



<https://olaw.nih.gov/home.htm>

PHS-Assured institutions will:

*"...establish mechanisms to document and monitor numbers of animals acquired and used, including any animals that are euthanized because they are not needed. Monitoring should not exclude the disposition of animals inadvertently or necessarily produced in excess of the number needed or which do not meet criteria (e.g., genetic) established for the specific study proposal. Institutions have adopted a variety of administrative, electronic, and manual mechanisms to meet institutional needs and PHS Policy requirements."*

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## Additional Information



For more information regarding:

- Housing
- Space allocation
- Per diems
- Transporting of animals
- Facility access
- Vet Care



Additional resources can be found:

- <https://web.jhu.edu/animalcare/>
- <https://researchanimalresources.jhu.edu/>

To schedule breeding training contact the ACUC: [acuc@jhmi.edu](mailto:acuc@jhmi.edu)

## Part II:

### Breeding Efficiency and Colony Management Software Presented by Transnetyx

